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Research Article

Interleukin-33 as a Potential Biomarker for Rheumatoid Arthritis: A Cross-Sectional Study

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease that affects about 1% of the general population. It is characterized by chronic, progressive, and systemic inflammation. Interleukin-33 (IL-33), is a pro-inflammatory cytokine believed to be involved in joint inflammation in RA. This cross-sectional research aims to determine whether interleukin-33 (IL-33) could serve as a potential biomarker for RA diagnosis and the evaluation of RA activity. The research involved 132 patients with inflammatory arthritis, and their serum levels of IL-33 and anti-citrullinated peptide antibody (ACPA) were measured using ELISA. Other routine biomarkers, including C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and rheumatoid factor (RF), were also measured. The median (IQR) of IL-33 was significantly lower in patients with RA [10.576 pg/mL (7.920)] compared to those with other types of inflammatory arthritis [12.896 pg/mL (5.700)]. The study also revealed a non-significant difference in IL-33 levels among the four disease activity groups according to DAS-28 ESR and DAS-28 CRP (P = 0.830, P = 0.340, respectively). Additionally, IL-33 showed a significant negative correlation with age (P = 0.019) and diabetes mellitus (P = 0.032). To the best of our knowledge, this study is the first to evaluate IL-33 as a diagnostic tool, showing a sensitivity of 59.8% and a specificity of 72% at a cut-off value of ≤11.8207 pg/mL. The IL-33 test alone is not sufficient for the diagnosis of RA or for differentiating it from other types of inflammatory arthritis. Furthermore, it cannot be used as a routine biomarker for evaluation of RA activity.

Keywords: *DAS-28 ESR; DAS-28 CRP; Interleukin-33; Rheumatoid Arthritis.*

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disorder that affects the synovial joints." It commonly starts in the small joints of the hands and feet but can also affect large joints symmetrically [1], [2]. Untreated RA may result in joint destruction and disability, significantly impacting a person's quality of life [3], [4]. Moreover, RA is associated with a higher mortality rate due to its extra-articular and cardiovascular manifestations [5].

The worldwide prevalence of RA is estimated to be around 0.5-1% [6]. Approximately 1% of the UK adult population is affected by RA [7]. In Iraq, a study found that the incidence of RA among patients visiting a rheumatology unit in Babylon Province increased from 1.60% in 2001 to 3.02% in 2011, with a cumulative incidence of 22.74% in 2011 [8]. Another study from 2014 to 2019 reported an increase in RA incidence from 1.1% in 2014 to 1.7% in 2019, with a cumulative risk of 10.0 [9].

Rheumatoid arthritis is a complex disorder characterised by a wide range of clinical manifestations and varying responses to treatment. Recent advancements in understanding the underlying pathogenesis of the disease have spurred increased interest in investigating biomarkers involved in different phases of RA [10]. These biomarkers play a crucial role in guiding the clinical and therapeutic management of the disease. They can help predict the development of RA in individuals at risk, improve diagnosis by closing the gap in serological testing, provide prognostic information for making treatment decisions and assessing treatment responses and outcomes, and allow for the monitoring of disease activity and progression [11].

When comparing RA diagnostic criteria, the significant role of biomarkers become evident [10]. In the "American College of Rheumatology (ACR) 1987 criteria, rheumatoid factor (RF) is the only included biomarker. However, the "ACR/EULAR 2010 criteria (American College of Rheumatology and the European League Against Rheumatism)" expanded the use of biomarkers by including four serological tests: ACPA, RF, ESR, and CRP [12], [13]. The biomarkers commonly used to diagnose RA have certain limitations. For instance, RF is present in 80% of RA patients but can also be found in healthy individuals and those with other diseases [14]. The ACPA test is more accurate, but both ACPA and RF still fail to detect 20-25% of seronegative RA patients. Furthermore, ESR is not specific and can be influenced by various factors [15]. Moreover, CRP levels can be normal in 40% of RA patients and elevated in other conditions [16]. Despite progress made with the inclusion of ACPA in the updated criteria, there is still a clear need for new biomarkers in the diagnosis of RA [17].

Interleukin 33 (IL-33), one of the cytokine members of the IL-1 family, acts as both a cytokine and a nuclear factor. It interacts with the ST2 receptor, leading to the activation of inflammatory cytokines. Additionally, IL-33 regulates inflammation and immunological responses, particularly Th2 immune responses [18], [19]. Studies have found a connection between IL-33 and autoimmune diseases such as Behçet's disease [20], ankylosing spondylitis [21], and RA. In RA, serum and synovial levels of IL-33 are elevated [22], [23]. The research aimed to investigate the potential use of IL-33 as a biomarker for the diagnosis of RA and to differentiate it from other types of inflammatory arthritis. Additionally, the study aimed to explore the role of IL-33 in evaluating RA activity.

Materials and Methods

From July 2023 to September 2023, a cross-sectional study was conducted on 132 patients suffering from inflammatory arthritis. The participants included those with RA as well as other types of inflammatory arthritis. All the patients who participated in the study were treated at the rheumatology department at Al-Sadr Medical City in Al-Najaf City. The study included 107 patients with RA and 25 patients with other types of inflammatory arthritis, such as systemic lupus erythematosus (15 patients), Sjögren's syndrome (3 patients), polymyalgia rheumatica (2 patients), Behçet's disease (2 patients), palindromic rheumatism (1 patient), psoriatic arthritis (1 patient), and monoarticular arthritis (1 patient). Among the participants, there were 121 females and 11 males, with ages ranging from 18 to 70 years.

The inclusion criteria involved patients aged between 18 and 70 years who were diagnosed with RA by a physician using the "2010 ACR/EULAR" diagnostic criteria for RA. Additionally, patients with other types of inflammatory arthritis were also included. Patients under 18 or over 70 years of age, as well as those with any other autoimmune diseases, central nervous system diseases, recent surgeries, wounds, acute local inflammation, chronic

infections, cancer, or immunodeficiency diseases, were excluded from the study.

For the study, inflammatory arthritis patients who visited the rheumatology department at Al-Sadr Medical City in Al-Najaf were randomly selected and divided into two groups. The first group comprised patients diagnosed with RA, who were classified into various levels of disease activity, such as "remission, mild, moderate, or severe", according to their DAS-28 ESR and DAS-28 CRP disease activity scores. The second group consisted of patients with different types of inflammatory arthritis other than RA [22].

All patients completed a questionnaire providing information on their name, age, gender, BMI, contraceptive pill use, family history of RA, as well as any history of chronic diseases like diabetes mellitus and hypertension, along with other relevant details. Additionally, a specialist conducted a physical examination of the patient's joints to assess the number of swollen and tender joints, which was involved in determining the disease activity score. Serum levels of IL-33 and ACPA in patients with inflammatory arthritis were evaluated using an ELISA kit (Sunlong, China). Furthermore, the sandwich immunodetection technique (Boditech, Korea) was used to evaluate RF, the Westergren method was employed to test ESR, and CRP was measured using a particle-enhanced immune-turbidimetry assay (Cobas, Germany).

Table 1. Demographic and clinical characteristics of the Inflammatory Arthritis Patients (n= 132)

Characteristic	RA patients (n=107)	Other inflammatory arthritis patients (n=25)	P- value
Demographics			
Age, mean (year) ± SD.	46.85 ± 10.564	39.48 ± 12.460	0.003**
Sex [females/males (% of females)]	98/9 (91.6%)	23/2 (92.0%)	0.999
BMI, mean (kg/m2) ± SD.	29.93 ± 5.424	27.40 ± 5.874	0.04*
Smoking [Smoker/Non-smoker (% of Smoker)]	10/97 (9.3%)	1/24 (4.0%)	0.152
Smoking index, mean pack per year ± SD.	15.24 ± 14.629	40.00 ± 0.0	0.141
Diabetes mellitus [Diabetic/Non-diabetic (% of Diabetic)]	17/90 (15.9%)	1/24 (4.0%)	0.005**
Contraceptive pill use [user/Non-user (Contraceptive pills user)]	6/91 (6.2%)	1/21 (4.5%)	0.744
Clinical data			
ESR level (mm/hr), median (IQR)	38.00 (30.00)	30.00 (32.50)	0.657
CRP level (mg/L), median (IQR)	5.49 (9.31)	1.68 (4.46)	0.01*
RF level (IU/mL), median (IQR)	18.00 (32.00)	8.00 (4.00)	0.001**
ACPA level (U/ml), median(IQR)	12.896 (5.042)	5.884 (2.572)	<0.001**
IL-33 level (pg/ml), median (IQR)	10.576 (7.920)	12.896 (5.700)	0.026*

Notes: BMI: Body mass index, SD: standard deviation, ESR: Erythrocyte sediment rate, CRP: C-reactive protein, RF: rheumatoid factor, ACPA: Anti-citrullinated peptide antibody, IL-33: Interleukin 33, IQR: Interquartile range. P< 0.05 (*), P < 0.01 (**).

Statistical analysis

The study utilized the Statistical Package for the Social Science (SPSS) software program, version 21, for data analysis. Continuous variables were presented as means and standard deviation

(SD), while categorical variables were expressed as frequencies and percentages. Various statistical tests were employed, including the Chi-square test, Pearson correlation, Spearman correlation, ANOVA, and independent *t*-test, to analyze different clinical and laboratory parameters among the research groups. Additionally, receiver operating characteristic (ROC) curves were used to evaluate the diagnostic utility of IL-33, RF, and ACPA in inflammatory arthritis patients by calculating the area under the curve (AUC), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). A significance level of $P<0.05$ was considered statistically significant, while $P<0.01$ was deemed highly significant.

Results and Discussion

A cross-sectional study was conducted on 132 patients with inflammatory arthritis, including 107 patients with RA and 25 patients with other types of inflammatory arthritis. The results showed a significant difference (P = 0.003) between the mean age of patients with RA (46.85 years ± 10.564) and those with other types of inflammatory arthritis (39.48 years ± 12.460). The mean BMI and SD of RA patients (29.93 kg/m2 ± 5.424) was significantly higher (P = 0.04) than that of patients with other types of inflammatory arthritis (27.40 kg/m2 ± 5.874).

There was no significant difference (P = 0.141) in the smoking index mean and SD between RA patients (15.24 ± 14.629) and patients with other inflammatory arthritis (40.00±0.0). Additionally, no significant differences were observed between the two groups in terms of sex, smoking, or contraceptive pill use (P = 0.999, P = 0.152, and P = 0.744, respectively). However, a significant difference (P = 0.005) was found regarding diabetes mellitus, as shown in table 1.

RA patients had higher median (IQR) ESR level [38.00 mm/hr (30.00)] than those with other types of inflammatory arthritis [30.00 mm/hr (32.50)]. However, this difference was not significant. In contrast, patients with RA had higher median (IQR) levels of CRP, RF, and ACPA [5.49 mg/L (9.31), 18.00 IU/mL (32.00), and 12.896 U/ml (5.042), respectively] compared to patients with other types of inflammatory arthritis [1.68 mg/L (4.46), 8.00 IU/mL (4.00), 5.884 U/ml (2.572), respectively]. The levels of these biomarkers differed significantly (P = 0.01, P = 0.001, and P = < 0.001, respectively) between the two patient groups. On the other hand, the median (IQR) IL-33 was lower in patients with RA [10.576 pg/mL (7.920)] compared to patients with other types of inflammatory arthritis [12.896 pg/mL (5.700)], with a significant difference (P = 0.026), as shown in table 1.

Table 2. Comparison of IL-33 According to the Disease Duration and Features of Treatment Intake

Characteristic	IL-33 mean ± SD (pg/ml)	P-value
Less than 6 months (n=28)	11.27± 6.272	0.850
More than 6 months (n=79)	11.12± 7.680	
Good response to treatment (n= 57)	10.09± 7.899	0.789
Poor response to treatment (n= 38)	11.28 ± 6.799	
Untreated newly diagnosed RA (n=12)	11.28 ± 6.150	0.795
Long-term regularly treated RA (n=54)	9.90±5.757	
Untreated newly diagnosed RA (n=12)	11.28 ± 6.150	0.788
Regularly treated newly diagnosed RA (n=13)	10.55 ± 7.256	

Interleukin 33 had a higher mean in RA patients with a disease duration of less than 6 months (11.27± 6.272) compared to those with more than 6 months of disease (10.12 pg/mL ± 7.680), but the difference was not significant (P = 0.850). Rheumatoid arthritis patients with a poor response to treatment had a higher mean of IL-33 (11.28 pg/mL ± 6.799) than those with a good response to treatment (10.09 pg/mL ± 7.899), but the difference was not significant (P = 0.789). Similarly, IL-33 had a higher mean in untreated newly diagnosed RA patients (11.28 pg/mL ± 6.150) compared to long-term, regularly treated RA patients (9.90 pg/mL ± 5.757) and regularly treated, newly diagnosed RA patients (10.55 pg/mL ± 7.256), but the difference was not significant, as shown in Table 2.

Table 3. Comparison of IL-33 Based on DAS-28 ESR Among Disease Activity Groups of RA Patients

Remission (n =2)	Low activity (n =2)	Moderate activity (n =33)	High activity (n =70)	P-Value
6.90 ± 9.379	9.106 ± 9.666	11.51 ± 5.678	11.18 ± 8.076	0.830

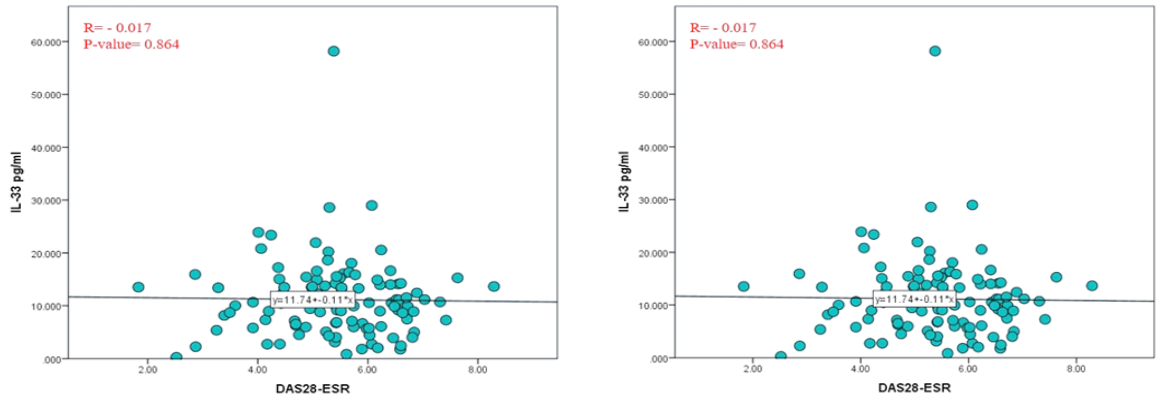
Notes: DAS-28 ESR of RA patients (n=107), IL-33 level (pg/ml), mean ± SD, DAS28 - Disease Activity Score, *ESR - Erythrocyte Sediment Rate.*

Table 4. Comparison of IL-33 Based on DAS-28 CRP Among Disease Activity Groups of RA Patients

Remission (n=6)	Low activity (n=6)	Moderate activity (n=61)	High activity (n=34)	P-Value
7.59 ± 6.200	9.21 ± 5.276	12.17 ± 8.367	10.33 ± 5.632	0.340

Notes: DAS-28 CRP of RA patients (n=107), IL-33 level (pg/ml), mean ± SD, CRP - C-reactive Protein, ESR - Erythrocyte Sediment Rate.

Additionally, there was no significant difference in IL-33 levels among the four disease activity groups according to DAS-28 ESR and DAS-28 CRP (P = 0.830, P = 0.340, respectively), as shown in table 3 and table 4. Furthermore, according to DAS-28 ESR and DAS-28 CRP, there was no significant correlation (R = -0.017, P = 0.864; R = 0.005, P = 0.962, respectively) between IL-33 levels and RA activity, as shown in Figures 1.



A B

Figure 1: Correlations of IL-33 with RA Activity Based on DAS-28 ESR (A) and on DAS-28 CRP (B).

Interleukin 33 showed a significant negative correlation with age (P = 0.019) and diabetes mellitus (P = 0.032). However, no significant correlation was observed

with sex, hypertension, smoking index, BMI, family history, disease duration, features of treatment intake, or contraceptive pill use (P > 0.05), as shown in Table 5. Additionally, IL-33 demonstrated no significant correlation with hemoglobin level, WBC count, or platelet count (P > 0.05), as shown in Table 6.

Table 5. Correlations of Demographic Characteristics, BMI and Features of Treatment Intake with IL-33 Values in the RA Patients

Characteristic of RA Patients		IL-33
Age (years)	r	-0.226
	P	0.019*
Sex	r	0.107
	P	0.273
Hypertension	r	-0.09
	P	0.357
Diabetes Mellitus	r	-0.207
	P	0.032*
Smoking index, (pack per year)	r	0.099
	P	0.312
BMI	r	-0.072
	P	0.455
Family History	r	0.006
	P	0.949
Disease Duration	r	-0.036
	P	0.709
Regularity of treatment intake	r	-0.062
	P	0.520
Response to Treatment	r	-0.019
	P	0.850
Untreated newly diagnosed RA/ treated long-term RA	r	-0.025
	P	0.806
Untreated, newly diagnosed/ Regularly treated, newly diagnosed RA	r	-0.066
	P	0.751
Contraceptive pill use	r	0.076
	P	0.457

*Notes: BMI body mass index, IL-33: Interleukin 33, r: Correlation coefficient, P: P-value. *: Significant difference.*

Table 6. Correlations of Haematological Parameters with IL-33 Values in the RA Patients

Characteristic of RA patients		IL-33
Haemoglobin level (mg/dl)	r	0.025
	p	0.802
WBC Count (x 10^9/liter)	r	0.006
	p	0.949
Platelets Count (x 10^9/liter)	r	-0.039
	p	0.69

Notes: WBC white blood cell, IL-33 interleukin 33 r correlation coefficient, P P-value.

Interleukin 33 had a very weak positive correlation with ACPA, but it was statistically not significant (P = 0.307). On the other hand, ESR had a statistically significant correlation with CRP (P < 0.001). Furthermore, CRP had a low positive correlation with RF, but it was statistically not significant (P = 0.059). However, RF had a highly statistically significant correlation with ACPA (P < 0.001), as shown in Table 7.

The diagnostic performance of IL-33, RF, and ACPA in distinguishing between RA and other types of inflammatory arthritis was evaluated using the Receiver Operator Characteristic (ROC) curve, as shown in Figure 2. The cut-off value for IL-33 was determined to be ≤11.8207 pg/mL, with an AUC of IL-33 was 0.618. Additionally, ACPA demonstrated very good diagnostic performance, with an AUC of 0.813, which was highly significant (P < 0.001). Rheumatoid factor also showed good diagnostic performance, with an AUC of 0.743, which was highly significant. However, IL-33 had a lower sensitivity (59.8 %) compared to RF (72.0 %) and ACPA (85.0%). Furthermore, IL-33 exhibited lower specificity (72.0 %) than RF and ACPA (84.0 % and 80.0 %, respectively), as shown in Table 8.

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Table 7. Correlations between Biomarkers in the RA patients

Parameters		IL-33	ESR (mm/h)	CRP (mg/L)	RF (IU/mL)	ACPA (U/ml)
IL-33	P	1				
	r	-				
ESR (mm/h)	r	-0.045	1			
	P	0.645	-			
CRP (mg/L)	r	-0.011	0.537	1		
	P	0.907	<0.001**	-		
RF (IU/mL)	r	-0.059	0.097	0.183	1	
	P	0.549	0.318	0.059	-	
ACPA(U/ml)	r	0.1	0.061	0.006	0.549	1
	P	0.307	0.534	0.949	<0.001**	-

*Notes: IL-33: Interleukin 33, ESR: Erythrocyte sediment rate, CRP: C-reactive protein, RF: Rheumatoid factor, ACPA: Anti-citrullinated peptide antibody, r: Correlation Coefficient, P: P-value. **: highly significant difference.*

Table 8. Receiver Operator Characteristic (ROC) Curve Characteristics of Inflammatory Arthritis Patients

Characteristic	IL-33	Rheumatoid Factor	ACPA
Area under the curve AUC	0.618	0.743	0.813
Standard error (SE)	0.056	0.063	0.053
Significant (Sig)	0.066	<0.001**	<0.001**
95% confidence interval	0.509-0.727	0.619 -0.867	0.709 -0.917
Optimal cut-point value (pg/ml)	11.8207	12.500	9.920
Sensitivity (%)	59.8 %	72.0 %	85.0 %
Specificity (%)	72.0 %	84.0 %	80.0 %
Positive predictive value (PPV)	90.1 %	71.96 %	85.04 %
Negative predictive value (NPV)	29.5 %	84 %	80 %
Diagnostic effectiveness (accuracy)	62.12 %	74.24 %	84.09 %
Youden's index	0.318	0.56	0.65

*Notes: IL-33: Interleukin 33, ACPA: Anti-citrullinated peptide antibody, **: Highly significant difference.*

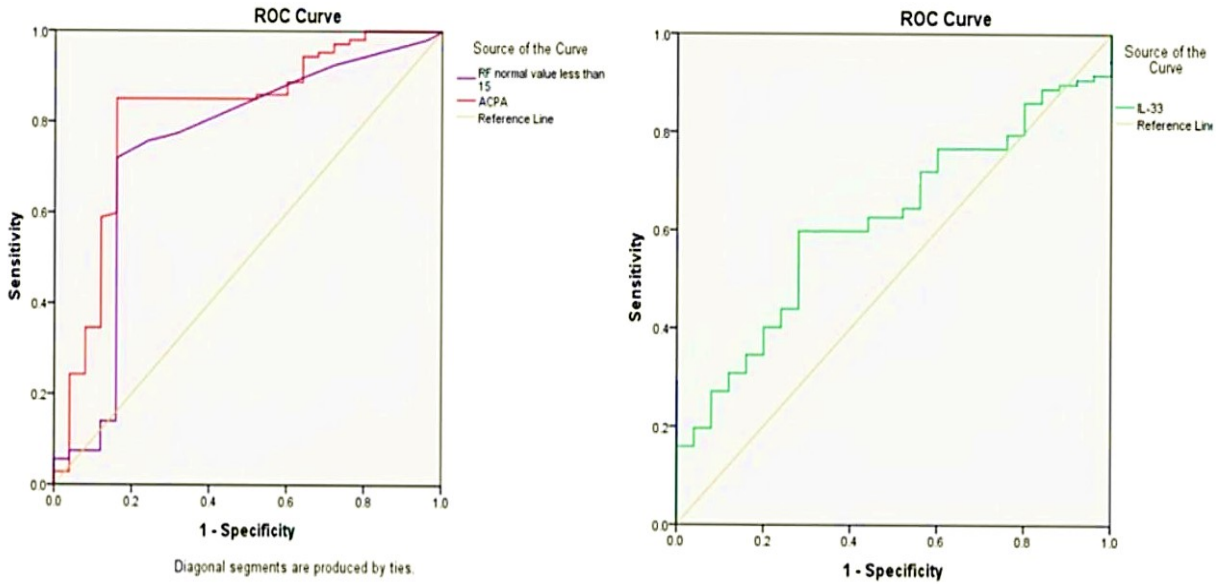

Figure 2: The Diagnostic Performance of RF, ACPA and IL-33.

Discussion

The findings of this study indicate that patients with RA had a significantly lower median IL-33 level than those with other types of inflammatory arthritis. This aligns with previous studies by Han *et al* [24], Dwedar *et al* [25], and Li *et al* [26], which reported elevated levels of IL-33 in patients with AS, SLE, and PsA, respectively. However, the present study's results contradict a study by Yang *et al* [27], which found that IL-33 levels in SLE patients were higher than in healthy controls but lower than those of RA patients.

These discrepancies may be attributable to differences in study populations, sample size, genetic and environmental factors, the sensitivity of the ELISA kits used for detection, or the differing roles and levels of IL-33 in each specific disease. Numerous studies have indicated that IL-33 levels correlate with the severity of inflammatory arthritis, suggesting that IL-33 and its receptor ST2 may be useful targets for predicting disease progression and improving therapeutic outcomes [28]. However, the lower levels of IL-33 in RA patients may be influenced by factors such as RA treatment, genetics, or inflammatory and immunological factors [29].

The current study found no significant difference in IL-33 levels based on disease duration in RA patients. This result is consistent with studies by Yang *et al* [27] and Elmahdy *et al* [30], which also found no significant correlation between IL-33 levels and disease duration. Conversely, Dwedar *et al* [25], observed a significant negative correlation between IL-33 levels and disease duration, with higher levels in patients with shorter disease duration. In contrast, Mu *et al* [31], reported that IL-33 correlated positively with disease duration, with higher levels in patients with longer disease duration. These conflicting results highlight the need for further research to clarify this relationship.

This study also found a non-significant difference in the median level of IL-33 concerning treatment response in RA patients, suggesting that treatment response does not significantly affect IL-33 levels. Intrestingly, the study observed a slightly higher median IL-33 levels in untreated, newly diagnosed RA patients compared to long-term, regularly treated RA patients. This result is consistent with the work of Yuan *et al* [19], who suggested that IL-33 has strong pro-inflammatory effects and found elevated IL-33 levels in the early phase of RA. One possible explanation for this difference is the effect of treatment on

IL-33 levels. Evidence indicates that cytokine-mediated immunity plays a role in the pathogenesis of autoimmune disorders like RA, potentially contributing to the observed elevated IL-33 levels.

Furthermore, IL-33 has demonstrated pro-inflammatory effects in human RA and experimental inflammatory arthritis [23]. It acts as an alarm signal for the immune system in response to infections, tissue damage, or necrosis, triggering various immune reactions [32]. However, the high levels of IL-33 in untreated, newly diagnosed RA patients compared to long-term, regularly treated RA patients did not reach statistical significance, possibly reflecting the complex and heterogeneous nature of RA.

The study also revealed a slightly lower level of IL-33 in newly diagnosed RA patients who were regularly treated compared to those who were untreated. However, this decrease did not reach statistical significance. One possible explanation for this decrease is the effect of RA medication on IL-33 levels, as mentioned earlier. Yuan *et al* [19], suggest that targeting IL-33 or its receptor could be a potential therapeutic approach for autoimmune diseases like RA. The above results are supported by Verri *et al* [33], Miller [34], and Matsuyama *et al* [35], who suggested that treatment can influence IL-33 levels in RA patients. For instance, anti-TNFα medications like infliximab may lower IL-33 receptor expression in neutrophils, leading to lower IL-33 levels and preventing them from migrating to the joint. Additionally, Hong *et al* [28], found that DMARDs therapy reduced IL-33 levels in RA patients, indicating that IL-33 could be a possible biomarker for inflammation.

The comparison of the findings on treatment response with the findings on treatment intake and its regularity on IL-33 levels suggests that the response to treatment may be more significantly affected by factors such as the type of therapeutic agents, genetic and environmental factors, rather than the regularity of treatment.

The research found that RA patients in the high activity group had a higher mean IL-33 level than those in other disease activity groups, based on disease activity as measured by the DAS-28 ESR and DAS-28 CRP indices. However, the remission group had the lowest mean IL-33, but this result was not statistically significant for both disease activity scoring methods by the DAS-28 ESR and DAS-28 CRP indices. This suggests a lack of association between IL-33 and disease activity, which is consistent with the findings of Mu *et al* [31], Xiangyang *et al* [36], Tang *et al* [21], Dwedar *et al* [25], and Choi *et al* [37]. However, other studies conducted by Matsuyama *et al* [35], Kageyama *et al* [38], Elmahdy *et al* [30], Shalaby *et al* [39], and Wang *et al* [40], reported a significant correlation between IL-33 and disease activity, where the serum level of IL-33 was higher in the high disease activity group.

The discrepancy between the results on disease activity and IL-33 levels suggests that the relationship between RA and these markers may not be straightforward. Further research is needed to determine whether IL-33 could serve as a potential biomarker to evaluate RA activity. This study found that IL-33 had a significant negative correlation with age and DM in RA patients.

The negative correlation between IL-33 and age suggests that younger RA patients may have higher serum IL-33 levels than older patients, which is consistent with previous studies by Mu *et al* [31], Yang *et al* [27], and Tang *et al* [21]. However, other studies by Hong *et al* [41], Dwedar *et al* [25], and Elmahdy *et al* [30], found no correlation between IL-33 and age in RA patients, indicating the need for further research to fully understand this relationship. Additionally, the negative correlation between IL-33 and age may be due to the long-term use of medications by older RA patients [42]. These medications can suppress the immune response and

lower the production of pro-inflammatory cytokines, including IL-33. Furthermore, other factors, such as hormonal and environmental factors, may contribute to higher levels of IL-33 in younger RA patients [23].

The study also found a significant negative correlation between IL-33 and DM, which is in line with previous research by Al-Rubaei *et al* [43] and Singh *et al* [44], which found lower IL-33 levels in patients with DM compared to the control group. The protective effect of IL-33 on reducing adiposity, improving glucose tolerance, and insulin resistance may explain this correlation [43].

The current study found no correlation between IL-33 and sex among RA patients, which is consistent with the results reported by Yang *et al* [27], Dwedar *et al* [25], and Elmahdy *et al* [30]. These studies also found no significant difference in IL-33 levels between males and females with RA. Additionally, the study found no significant correlation between IL-33 levels and the duration of RA disease, which is consistent with Choi *et al* [37] and Elmahdy *et al* [30]. However, Mu *et al* [31] and Shalaby *et al* [39], observed a significant positive correlation between IL-33 and disease duration in RA patients, while Dwedar *et al* [25], reported a significant negative correlation between IL-33 levels and disease duration in RA patients.

The study found no significant correlation between IL-33 and various factors such as hypertension, smoking index, BMI, family history, response to treatment, features of treatment intake, and contraceptive pill use. Interestingly, the researchers also noted that there were no previous studies available for comparison with these results, highlighting the novelty of their findings. This lack of correlation suggests that IL-33 may not be influenced by these common risk factors, opening the door for further research to explore its role in other pathways or conditions [45].

Furthermore, the study found a non-significant correlation between IL-33 and hematological parameters such as hemoglobin, WBC, and platelets in patients with RA. In this regard, the current study's findings align with those of Kageyama *et al* [38], Duan *et al* [46], Dwedar *et al* [25], and Elmahdy *et al* [30], which also reported a non-significant correlation between IL-33 serum levels and hemoglobin or platelet counts in RA patients. However, the results of the current study contradict previous studies conducted by Kageyama *et al* [38] and Elmahdy *et al* [30], who found a significant correlation between IL-33 and WBC count in RA patients.

Additionally, the study found a non-significant correlation between IL-33 and routine biomarkers, including ESR, CRP, RF, or ACPA, in patients with RA. Regarding the non-significant correlation between IL-33 and ESR, the present study's findings align with previous studies conducted by Mu *et al* [31], Xiangyang *et al* [36], Tang *et al* [21], and Choi *et al* [37], which also found a non-significant correlation between IL-33 and ESR. However, Yang *et al* [27], and Elmahdy *et al* [30], found a significant positive correlation between IL-33 and ESR.

Regarding the non-significant correlation between IL-33 and CRP, the present study's findings are consistent with previous studies conducted by Mu *et al* [31], Hong *et al* [41], Xiangyang *et al* [36], and Tang *et al* [21]. However, other studies, including Yang *et al*, [27], Kageyama *et al* [38], Al-Rubaei *et al* [43], Elmahdy *et al* [30], Shalaby *et al* [39], and Wang *et al* [40], found a significant positive correlation between IL-33 and CRP.

Regarding the non-significant correlation between IL-33 and RF, the present study's findings contradict previous research conducted by Mu *et al* [31], Xiangyang *et al* [36], Khalifa *et al* [18], Tang *et al* [27], Choi *et al* [37], Elmahdy *et al* [30], and Wang *et al* [40], which found a significant positive correlation between IL-33 and RF. However, the current study's non-significant correlation between IL-33 and ACPA is consistent with Tang *et al*

[21], while Mu *et al* [31], Elmahdy *et al* [30], and Rivière *et al* [47], found a significant positive correlation between IL-33 and ACPA.

The current study used receiver operating characteristic (ROC) curve analysis to evaluate the value of RF, ACPA, and IL-33 in the diagnosis of RA and differentiating it from other types of inflammatory arthritis. This study is the first to use ROC curve analysis to measure the diagnostic value of IL-33 and its usefulness in distinguishing RA from other types of inflammatory arthritis. The AUC for IL-33 was 0.618 with no significant P value at a cut-off value of ≤11.8207 pg/ml, with IL-33's sensitivity and specificity being 59.8% and 72.0%, respectively.

Compared to ACPA and RF, IL-33 had a lower AUC, sensitivity, specificity, and accuracy. The effectiveness of IL-33 as a diagnostic biomarker for RA is uncertain, and the present study's findings differ from Elmahdy *et al* [30], which found that IL-33 had excellent diagnostic accuracy with a sensitivity of 98% in distinguishing RA from healthy controls.

Conclusions

In conclusion, this study suggests that IL-33 is not a reliable biomarker for RA diagnosis or disease activity evaluation. Further research is needed to identify more effective biomarkers for the diagnosis and management of RA.

Approval of the Ethical Committee

The study project was approved by the ethical committee of the College of Medicine at the University of Kufa before it began. Furthermore, permission was granted from the Rheumatology Department of Al-Sadr Medical City, and the patient's consent was also obtained to conduct a questionnaire and collect a blood sample.

Conflict of Interest

We declare that there is no conflict of interest.

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